A Non-Heme Iron Protein from Pig Testis and Its Substitution for Adrenal Non-heme Iron Protein (Adrenodoxin) in Steroid 11β-Hydroxylation*

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Received February 6, 1967

In a series of studies on pig adrenal steroid hydroxylases from the mitochondrial fractions, it has been found that a non-heme iron protein, adrenodoxin, is one of the components of the electron transfer system of steroid 11β-hydroxylase; and that this protein contains 2 iron atoms and 2 moles of labile sulfide per mole, has a molecular weight of approximately 20,000, has a high (positive) oxidation-reduction potential and shows the signal of electron spin resonance at g=1.94 upon reduction (Suzuki and Kimura, 1965; Kimura and Suzuki, 1965; Watari and Kimura, 1966; Kimura and Suzuki, 1967). Also, a protein similar to adrenodoxin was isolated from bovine adrenal mitochondrial fractions by Omura et al. (1965).

This communication describes the existence of a non-heme iron protein obtained from pig testis and similar to adrenodoxin. The substitution of this protein (testodoxin)*** for adrenodoxin in adrenal steroid 11β-hydroxylation is reported.

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* This study was supported in part by a grant (AM-09243) from the National Institutes of Health, United States Public Health Service, and by funds from the Ministry of Education in Japan.

** Special research fellow.

*** The trivial name testodoxin is proposed for the non-heme iron protein from testis.

600 g of acetone powder prepared at -20° from about 6 kg of frozen pig testis was homogenized with 3000 ml of 0.15 M KCl containing 0.01 M phosphate buffer (pH 7.4) at the low speed of a Waring blendor. After filtration with cheese cloth, the filtrate was incubated at 38° for one hour with naja-naja snake venom (1.0 mg venom per 100 g of the acetone powder). The mixture was then centrifuged at 12,000 x g for 20 minutes. The supernatant solution was fractionated by the addition of solid ammonium sulfate. The fraction precipitated at 40 to 80 % saturation was dialyzed against 0.01 M phosphate buffer (pH 7.4). The dialyzed solution was placed into a DEAE-cellulose column (4.2 cm x 20 cm), which had been equilibrated with 0.01 M phosphate buffer (pH 7.4). After complete washing with the same buffer, testodoxin was eluted with 0.50 M KCl containing 0.01 M phosphate buffer (pH 7.4). The eluate was concentrated by ammonium sulfate precipitation. After dialysis, the concentrated solution was further purified by passing it through a Sephadex G-75 column (1.0 cm x 24 cm), equilibrated with 0.01 M phosphate buffer (pH 7.4). Further purification was achieved by a second DEAE-cellulose column.

The purified material displayed visible absorption maxima at 415 mμ and 455 mμ, which are exactly the same as those of adrenodoxin (Fig. 1). The spectrum is typical of a non-heme iron protein. Analysis for non-heme iron by the method of Massey (1957) and for labile sulfide by the method of Fogo and Popowsky (1949), slightly modified, revealed that this purified material contained equimolar amounts of non-heme iron and labile sulfide. It must be mentioned here that the preparations still were impure judging from the low iron content, if we assume that testodoxin has the same iron content as adrenodoxin. However, there was no

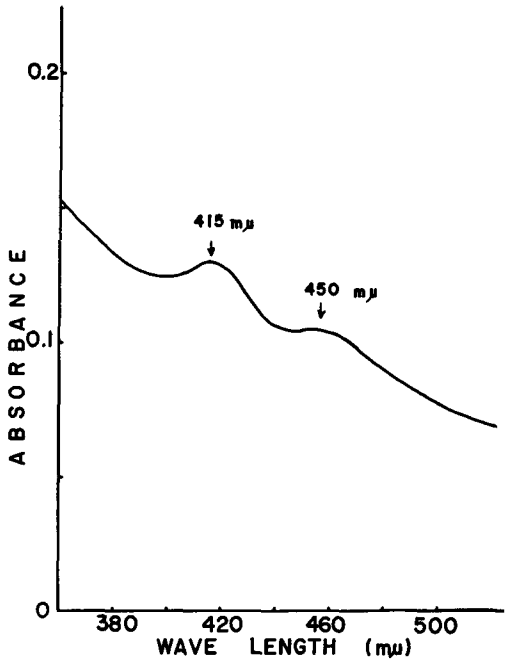

Fig. 1, Visible Spectrum of Pig Testis Non-Heme Iron Protein (Testodoxin)

The solution contained testodoxin (3.84 mg protein) in 1.50 ml of 0.1 M tris buffer (pH 8.8).

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flavin and no heme detected by fluorometric and spectrophotometric measurements.

As shown in Fig. 2, the formation of corticoid depended on the amount of testodoxin added in the presence of the adrenal preparation containing NADPH dehydrogenase (adrenodoxin reductase) and cytochrome P_{450} particles. It can be concluded that testodoxin served as an oxidation-reduction component in the electron transfer system of adrenal steroid hydroxylase. Testis steroid hydroxylase appears to consist of a flavoprotein, a non-heme iron protein (testodoxin) and cytochrome P_{450}. The sequence in the order given is apparently the same as that for adrenal

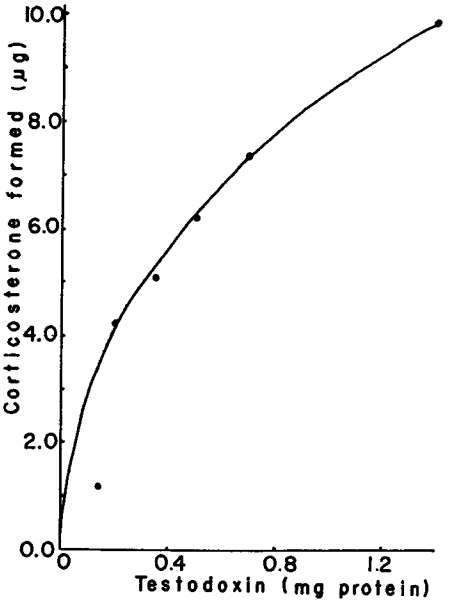

Fig. 2, Substitution of Testodoxin for Adrenodoxin in Adrenal Steroid 11β-Hydroxylation

The reaction mixture had: 1 μmole of deoxycorticosterone in propylene glycol, 60 μmoles of tris buffer (pH 7.4), 10 μmoles of $MgCl_2$, 50 μmoles of nicotinamide, 1 μmole of mercaptoethanol, 1.9 μmoles of NADP, 5 μmoles of glucose-6-phosphate, 0.10 ml (0.08 μmole of NADPH formed per min) of yeast glucose-6-phosphate dehydrogenase. After shaking for one hour at 37° in air phase, corticosterone formed was extracted with dichloromethane and the quantity was estimated by the fluorometric method of Mattingly (1962) with minor modifications.

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mitochondrial steroid hydroxylases, which has been proposed by Omura et al. (1965) and by Kimura (1966 a,b).

The content of testodoxin in the testicular gland was preliminarily compared with that of adrenodoxin in the adrenal gland. The results showed that the adrenal had about 10 times as much non-heme iron and labile sulfide as the testis had in the extracts with 0.15 M KCl from the respective acetone powders. Furthermore, we could not find any indication that liver microsomes contain any non-heme iron protein like adrenodoxin. It may

be of interest to mention that either a similar extract or the partially purified preparation from the ovaries was able to be substituted for adrenodoxin. At the present time, it seems reasonable to postulate that the endocrine organs with steroid hormone biosynthesis may have non-heme iron proteins very similar to adrenodoxin, and that the electron transfer systems in their steroid hydroxylases resemble each other fairly well. In this connection, Billiar et al. (1966) recently found that placental extracts can substitute for adrenal factors in the steroid 11β-hydroxylation, although it was not clear whether their factors are non-heme iron protein or not.

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